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Analysis of the Human Serological Immune Response to a Variable Region of the Attachment (G) Protein of Respiratory Syncytial Virus During Primary Infection

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The serum antibody responses of babies to the variable carboxy-terminal region of the attachment (G) protein of respiratory syncytial virus (RSV) have been analysed using paired acute and convalescent sera from infants experiencing their first RSV infection with viruses of known genotype. The variable 84–85 carboxy-terminal amino acids of the G protein of six recent isolates of group A RSV were expressed in *Escherichia coli* as fusion proteins with glutathione S-transferase. About half the infants developed antibodies which recognised these fusion proteins. The patterns of response obtained in enzyme linked immunosorbant assays and immunoblotting assays were closely related to the infecting genotype.

KEY WORDS: attachment (G) protein, respiratory syncytial virus, human antibodies

INTRODUCTION

Respiratory syncytial virus (RSV) is a major cause of bronchiolitis in babies and infants. The virus is unusual in that it can repeatedly re-infect individuals and infect babies in the presence of maternal antibody. RSV isolates can be divided into two major groups, A and B, and these groups can be further subdivided into a number of genotypes by nucleotide sequencing and reaction of isolates with monoclonal antibodies [Anderson et al., 1985; Mufson et al., 1985; Gimenez et al., 1986; Johnson and Collins 1989; Cane and Pringle, 1991]. The attachment (G) protein gene has been found to show the greatest variability both between and within the groups; for example, the G protein of two prototype A and B group strains show only 53% amino acid identity, while within group A there is up to 20% amino acid variability and within group B 9% amino acid variability [Johnson et al., 1987; Cane et al., 1991; Sullender et al., 1991]. In addition, there is considerable antigenic diversity seen with respect to the G protein [Storch and Park, 1987; Garcia-Barreno et al., 1989] and this has been found to correlate with the division of the isolates into genotypes on the basis of nucleotide sequences [Garcia et al., 1994; Cane and Pringle, 1995]. The variability of the G protein is concentrated in two parts of the ectodomain of the protein separated by a highly conserved region. There is evidence to suggest that these variable regions show progressive accumulation of amino acid changes which may be correlated with antigenic change [Cane and Pringle, 1995].

There have been a number of studies which have addressed the question of the location of antigenic sites on the G protein. The G proteins of subgroup A RSV isolates are between 289 and 298 amino acids long with an external carboxy terminus which is extensively glycosylated with both O- and N-linked sugars. Norrby et al. [1987] analysed the reactions of rabbit hyperimmune antisera, human convalescent sera, and murine monoclonal antibodies (mabs) with overlapping peptides representing the ectodomain of the protein. They found seven peptides representing amino acids 84–98, 94–108, 134–148, 144–158, 174–188, 194–208, and 224–238 that reacted with the rabbit sera, three peptides that reacted with the human sera (144–158, 174–188, and 184–198), and one peptide that reacted with the mouse mabs (174–188). None of these peptides, with the exception of some recognised by the rabbits, correlated with the variable regions on the protein, especially the carboxy terminus. However, a possible explanation for the failure of the human sera to react with the variable regions of the protein is

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that these sera did not contain antibodies to the particular variable region sequence present in the prototype A2 strain of group A, from which the peptides were derived.

Sullender [1995] used chimaeric G proteins derived from a mix and match of three regions (amino acids 1–173, 174–214, and 215–end) from group A and B RSV strains and found that all three regions contributed to recognition by G specific mabs. However, by expressing a G protein truncated at amino acid 230, it was shown that this construct could provide just as much protection against viral challenge in experimental animals as the full length protein [Olmsted et al., 1989], indicating that the terminal 69 amino acids might be irrelevant to protective immunity.

Some mouse mabs to the G protein show variable reactions with isolates, and these have been used to generate escape mutants. The majority of the escape mutants were found to have mutations in the variable 3′ *end of the* gene [Rueda et al., 1991]. One escape mutant was found to have a frame shift mutation which changed the carboxy terminal one third of the protein and concurrently lost reactivity with many other mabs and a polyclonal antiserum raised against affinity purified G protein [Garcia-Barreno et al., 1990]. These results demonstrate that this carboxy-terminal variable region includes antigenic areas. However, competition ELISAs between human convalescent sera and murine mabs failed to locate any reaction of the human sera to this region [Palomo et al., 1991]. However, since the genotypes of RSV infecting babies are now known to be highly variable, their immune response to these variable regions might only be detectable if the target antigen accurately reflected the infecting virus.

We attempted to address the problem of analysing the human immune response to the variable regions of the G protein by utilising paired acute and convalescent sera from infants experiencing their first RSV infection with a virus of known genotype. To facilitate this, the variable carboxy termini from the G proteins of virus isolates representing each of the currently circulating genotypes of group A RSV have been expressed as fusion proteins with glutathione-S-transferase (GST) in *Escherichia coli* (*E. coli*). The serum antibody responses shown by the babies to each of these expressed polypeptides have been analysed and demonstrate that infants can exhibit responses to the G protein of RSV specific to the infecting genotype.

MATERIALS AND METHODS

Patients

Between 1 January 1991 and 31 March 1992, all infants undergoing investigation by sampling of nasopharyngeal aspirate (NPA) for virological analysis after admission with respiratory disease at the Royal Liverpool Children's Hospital, Liverpool, UK, were prospectively enrolled into a study of RSV infections. Ethical committee approval for the study permitted additional samples to be obtained including acute and convalescent sera. Confirmation of RSV infection was by immunofluorescence tests on NPAs or on cell culture passaged virus. Forty-seven patients from the first epidemic are detailed in Table I, and 15 patients from the second epidemic in Table II.

Genotyping of Infecting Virus

The genotypes of the RSV infecting the babies were determined as previously described [Cane and Pringle, 1992]. Briefly, parts of the N and G genes from either NPAs or cultured virus were amplified by PCR and the PCR products analysed by restriction mapping. In some cases, genotype designation was confirmed by nucleotide sequencing of variable regions of the G gene. In the case of group A isolates these genotypes have been previously designated SHL1-6. However, SHL1, 3, and 4 isolates have been found to have extremely similar G gene sequences and restriction patterns so these have not been distinguished for this study: isolates belonging to this genotype will be designated SHL1/3/4 for this report. B group isolates were divided into NP1 and NP3 genotypes.

Expression of a Variable Part of RSV G Protein

The virus strains chosen for expression of a variable part of the G protein were group A isolates from 1989–90 in Birmingham, UK, for which the entire G gene sequences have been determined [Cane et al., 1991; our unpublished data]. These isolates were RSB89-642 (genotype SHL5), RSB89-5857 (SHL1), RSB89-6190 (SHL2), RSB89-6256 (SHL3), RSB89-6614 (SHL4), and RSB90-8106 (SHL6). The relatedness of the G proteins of these isolates is illustrated in Figure 1.

RNA extraction and cDNA synthesis were carried out as previously described [Cane et al., 1991; Cane and Pringle, 1992]. The terminal 3′ end of the G genes was then amplified using PCR with the following primers: Primer 1, 5′(GCGGATCCT)AGATCACAAACCTCAA-AC3′, which corresponds to nucleotides 654–671 of RSB89-6190; Primer 2, 5′(GGAATTCGTCGAC)TTTT-TTTTTTTTTTAAT3′, which is complementary to the polyA tail together with the terminal 3 nucleotides of the G gene message; and Primer 3, 5′(GGAATT)CTGG-TTTGTTATGTTGGATGG3′, which is complementary to nucleotides 889–909 of RSB89-6190. The parentheses indicate additional nucleotides to provide restriction sites. Primers 1 and 2 were used for all isolates except RSB89-6190 for which primers 1 and 3 were used. PCR products were cloned into the *Bam*HI and *Sal*I sites (RSB89-642, -5857, -6256, -6614, and 90-8106), and *Bam*HI and *Eco*RI sites (RSB89-6190) of Bluescribe (Vector cloning systems) and then subcloned using the same restriction sites into the GST gene fusion vector pGEX-5X-3 (Pharmacia). Expression and purification of the fusion proteins by affinity chromatography using glutathione sepharose (Pharmacia) were carried out according to the manufacturer's protocols. This allowed the expression of the terminal 84–85 amino acids of the G proteins from these isolates. In the case of RSB89-6190, the termination codon was not included in the PCR primer so, in this construct only, the termination was provided by the pGEX plasmid after addition of a further 12 amino acids. The authenticity of the plasmid

TABLE I. Patients Infected With RSV During First Epidemic

Study no.	Age	Infecting RSV group:genotype	Time between serum samples	Increase in serum response to fusion proteins in ELISA tests SHL1/3/4	SHL2	SHL5	SHL6
3	5w	A:1/3/4	2w	0[a]	0	0	0
4	9w	A:ND[b]	18w	+/−	++	0	+/−
7	4w	A:1/3/4	11d	0	0	0	0
8	19w	A:1/3/4	10w	+++	++	++	++
9	14w	A:ND	10d	+/−	0	0	0
10	15w	A:1/3/4	10w	0	0	0	0
11	4w	A:1/3/4	18w	0	+/−	0	0
19	8w	A:5	11w	0	0	++	0
20	9w	A:1/3/4	18w	0	0	0	0
23	16w	B:NP1	16w	0	0	0	0
24	14w	A:6	16w	+/−	+/−	+/−	+
25	15w	A:6	16w	+	++	+	++
27	31w	A:1/3/4	10w	++++	++++	+++	+++
31	25w	A:1/3/4	2w	0[c]	+/−[c]	0[c]	0[c]
33	2w	A:1/3/4	2w	0	0	0	0
34	14w	A:1/3/4	2w	0	0	0	0
35	31w	A:ND	2w	+++	++	0	+++
40	17w	A:6	13d	0	0	0	+/−
43	11w	A:ND	10w	+	++++	0	+
44	8w	A:1/3/4	3w	0	0	0	0
45	9w	A:1/3/4	2w	0	0	0	0
47	29w	A:1/3/4	2w	++++	++++	++++	++
50	24w	A:5	10w	0	0	+	0
54	18w	A:ND	7d	0	++	0	+
57	20w	A:1/3/4	2w	0	0	0	0
58	9w	A:1/3/4	10w	+	0	+/−	−
59	3w	ND	2w	0	0	0	0
61	17w	A:6	12w	0	0	0	0
69	15w	A:2	10d	0	+++	0	0
79	10w	A:1/3/4	2w	0	0	0	0
86	11w	A:1/3/4	12d	+	+	+/−	+/−
88	6w	A:1/3/4	ND	0[d]	0[d]	0[d]	0[d]
91	2w	A:6	12w	0	0	0	0
96	7w	A:2	7d	0	+	0	0
98	9w	A:6	7d	+/−	+	0	++
99	7w	A:ND	7d	0	0	0	0
104	18w	B:NP3	3w	0	0	0	+/−
107	44w	A:2	5d	+/−	+++	0	+/−
109	24w	A:2	10w	0	++++	0	0
111	4w	A:1/3/4	ND	0[d]	+[d]	0[d]	0[d]
114	8w	A:1/3/4	5w	0	0	0	0
125	13w	A:1/3/4	ND	0[d]	0[d]	0[d]	0[d]
127	52w	A:ND	ND	0[d]	++++[d]	+/−[d]	+/−[d]
136	5w	A:1/3/4	ND	ND	ND	ND	ND
165	24w	A:ND	ND	+++[d]	++++[d]	+/−[d]	+[d]
178	6w	A:1/3/4	3w	0	0	0	0
203	37w	A:1/3/4	ND	+++[d]	++[d]	+[d]	+[d]

[a]0, no rise or fall.
[b]ND, not determined.
[c]High acute serum levels.
[d]Acute serum not tested; level in convalescent serum.

constructs was checked by nucleotide sequencing. Correct expression of the G gene sequences was checked by reaction of the fusion proteins with G-specific mabs 021/9G and 25G [Garcia et al., 1994] kindly provided by Dr J. A. Melero, Instituto de Salud Carlos III, Madrid, Spain.

Enzyme-Linked Immunosorbant Assay

Purified fusion proteins were coated on to 96-well plates (Immulon 2) using carbonate coating buffer. The plates were incubated overnight at 4°C, then blocked with 5% dried milk in PBS with 0.1% Tween (PBS-T) at 37°C for 30 min. Antibody was diluted in the blocking mix and incubated with the antigen for 1.5 hr at 37°C. Following five washes with PBS-T using a LabSystems automatic platewasher, the plates were incubated with goat anti-human IgG (H&L) conjugated with horseradish peroxidase (Biorad), then washed extensively and developed using o-phenylenediamine dihydrochloride. Absorbances were read at 492 nm. Where the reactions of fusion proteins with mabs were being examined, the second antibody was replaced with a goat anti-mouse IgG conjugate (Biorad).

Immunoblotting

Purified fusion proteins were run on 12% polyacrylamide gels under reducing conditions. The proteins were

TABLE II. Patients Infected With RSV During Second Epidemic

Study no.	Age	Infecting RSV group:genotype	Time after admission of convalescent serum sample	Level of convalescent serum reaction with fusion proteins in ELISA tests			
				SHL1/3/4	SHL2	SHL5	SHL6
260	15w	A:ND[a]	19w	0	++	0	0
268	5w	A:ND	19w	0	+/−	0	0
293	36w	A:2	22w	+/−	++	0	+
323	16w	A:ND	21w	+/−	++	0	+/−
451	42w	A:2	21w	+	+++	+	+
478	24w	A:ND	21w	++	++++	+	++
495	13w	A:2	21w	0	++++	0	0
536	6w	A:2	22w	0	+/−	0	0
545	8w	A:2	26w	+/−	++	0	+
586	34w	A:2	22w	+/−	+++	0	+/−
691	28w	A:ND	21w	++	++++	0	0
706	39w	A:2	21w	++	++++	+	++
716	5w	A:2	25w	0	+++	0	0
735	19w	A:ND	21w	++	++++	+	++
785	6w	B:NP3	ND	ND	ND	ND	ND

[a]ND, not determined.

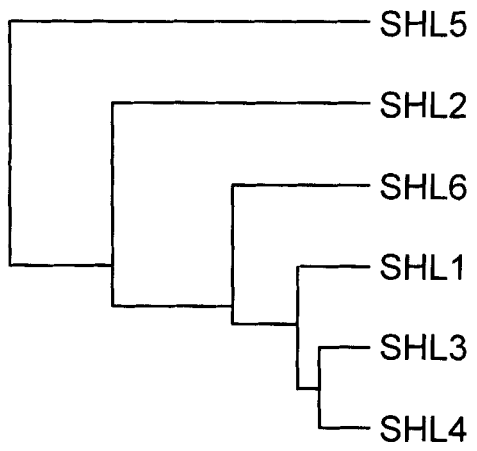


Fig. 1. Diagram to illustrate the relatedness of the G proteins from the various genotypes of group A RSV [based on Cane et al., 1991, and unpublished data].

then transferred to nitrocellulose membranes by standard methods. The blots were blocked using 5% dried milk in PBS-T, then incubated with antibody diluted in blocking mix for 1.5 hr at room temperature. Following extensive washing the blots were incubated with goat anti-human IgG peroxidase conjugate (Biorad), then washed extensively and developed using 2,4-diaminobenzidine.

RESULTS

Expression of 3′ End of RSV G Gene as a GST Fusion Protein in *E. coli*

The carboxy-terminal 84–85 amino acids of the G proteins from subgroup A RSV isolates RSB89-642 (SHL5), RSB89-5857 (SHL1), RSB89-6190 (SHL2), RSB89-6256 (SHL3), RSB89-6614 (SHL4), and RSB90-8106 (SHL6) were expressed as soluble GST fusion proteins in *E. coli*. The fusion proteins were designated GST-G3′-642, GST-G3′-5857, GST-G3′-6190, GST-G3′-6256, GST-G3′-6614, and GST-G3′-8106 respectively. About 0.5 mg of fusion protein were obtained per litre of bacterial culture following affinity purification using glutathione-sepharose. The eluate from the sepharose was analysed by SDS-polyacrylamide electrophoresis followed by Coomassie blue staining. Typical preparations are illustrated in Figure 2. It can be seen that the fusion proteins migrated with the predicted mobilities, and were highly purified from other bacterial proteins with the exception of one band at about 80 kD. In many of the preparations there appeared to be some proteolytic breakdown of the fusion proteins, giving in particular a band of mobility of approximately 30 kD (Fig. 2). The expression of the G derived polypeptides was checked by the reaction of the fusion proteins with either mab 25G (GST-G3′-642) or mab 021/9G (GST-G3′-5857, -6190, -6256, -6614, and -8106) in both immunoblotting and ELISA tests (not shown).

Genotyping of Infecting Virus

RSV isolates or clinical specimens (NPAs) from 57 babies were classified into RSV genotypes by PCR and restriction mapping of parts of the G and N protein genes. Thirty-eight samples were analysed from the first epidemic studied and 20 from the second. The first epidemic showed a wide mixture of genotypes: 24 samples belonged to SHL 1/3/4, 6 belonged to SHL6, 4 to SHL2, and 2 to SHL5: these are all group A genotypes. Two specimens from the first epidemic were group B, one each of NP1 and NP3 genotypes. A further eight samples were determined to be subgroup A but not further classified. In contrast, the second epidemic was much more homogeneous with 19 of 20 specimens being classified as SHL2 and the remaining sample being a group B isolate, NP3; 6 other samples were classified as group

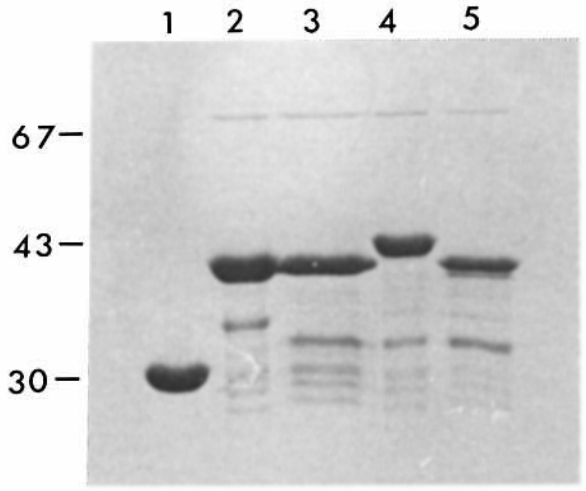


Fig. 2. SDS-PAGE analysis of GST-fusion proteins derived from 84–85 carboxy terminal amino acids of the G protein from group A isolates of RSV. **Lane 1,** unfused GST; **lane 2,** GST-G3′-642; **lane 3,** GST-G3′-5857; **lane 4,** GST-G3′-6190; **lane 5,** GST-G3′-8106. Mobility of size standards is shown to the left.

A only on the basis of N gene analysis. The genotype of RSV infecting the babies is shown in Tables I and II.

Immune Responses to Bacterially Expressed G Protein Fragments

Forty pairs of acute and convalescent sera from the first epidemic were tested by ELISA against the purified GST-G3′ fusion proteins. Due to the extremely small amounts of sera available, the sera were tested initially at one concentration only (1 in 200), and changes deduced from alterations in O.D. values rather than from titres. Twenty-two pairs of sera showed no change or an equivocal increase in reaction or a reduction in their reaction with the fusion proteins. These included the two pairs from the group B infected patients for which the appropriate antigens were not available. Eighteen convalescent sera showed a marked rise in reactivity with at least one of the fusion proteins relative to the paired acute sera. The results of the ELISA test for each baby are shown in Table I. The average age of the low or non-responding babies (excluding those infected with group B viruses) was 10 weeks (range 2–25 weeks) while that of the responders was 18.2 weeks (range 7–44 weeks); no baby under 7 weeks of age showed an unequivocal response in these tests.

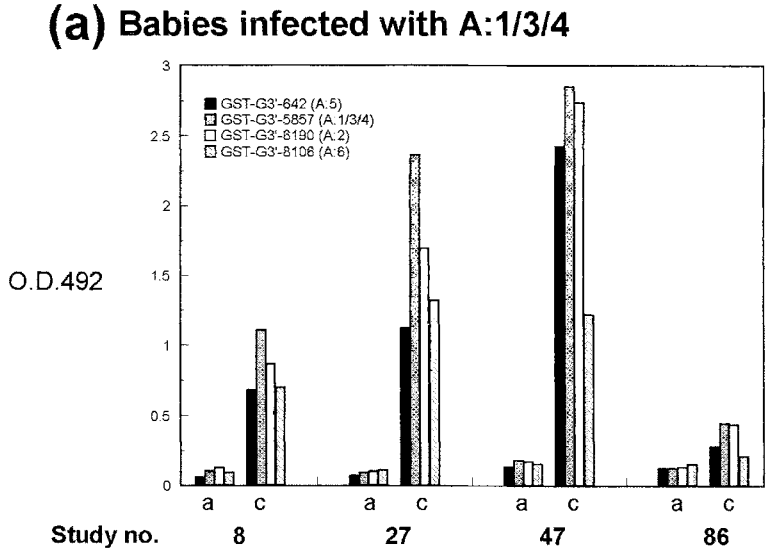
A typical result of an ELISA test is illustrated in Figure 3. This figure shows the O.D.s given by an ELISA by 12 pairs of sera tested against GST-G3′-642, GST-G3′-5857, GST-G3′-6190, and GST-G3′-8106. None of these baby sera showed a significant reaction with the unfused GST protein (data not shown). The responses of babies to GST-G3′-5857, GST-G3′-6256, and GST-G3′-6614 (representing SHL1, 3, and 4) were similar (data not shown), so the data have been combined for these genotypes. The majority of the acute sera showed little or no reaction with the fusion proteins, except in one case (study no. 31) where very strongly reacting antibodies were detected in both acute and convalescent sera.

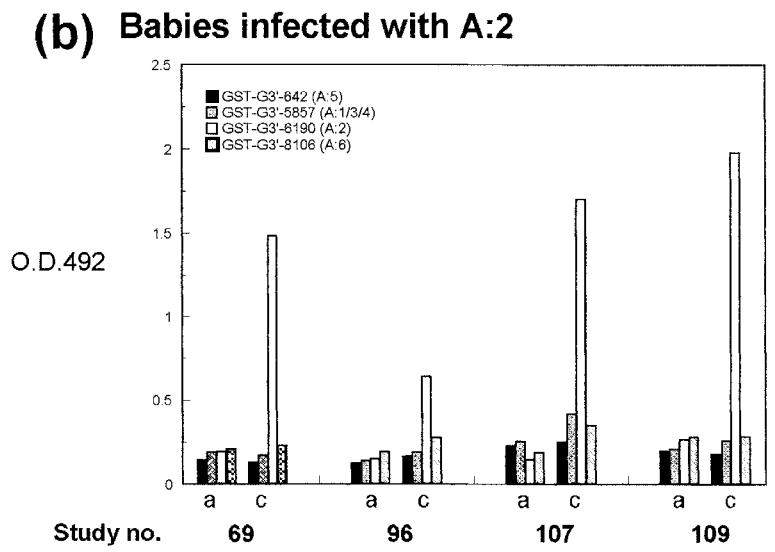
Figure 3 and Table I show that the specificity of the babies' serological responses to the fusion proteins varied with the genotype of the infecting virus. The convalescent sera from the 5 of 18 babies infected with SHL1/3/4 (study nos. 8, 27, 47, and 86 illustrated in Figure 3a) and the 3 of 6 babies infected with SHL6 viruses (study nos. 25 and 98 illustrated in Figure 3c) who showed a response were largely cross-reactive with all of the fusion proteins although the greatest response was in general seen against the fusion protein derived from the homologous genotype. In contrast, both babies infected with SHL5 viruses (study nos. 19 and 50) showed responses to their homologous antigen, namely GST-G3′-642, with only a slight, if any, response to the other fusion proteins. The most specific responses were observed in all of the four babies infected with SHL2 type virus (study nos. 69, 96, 107, and 109). These all gave a very strong and highly specific response to GST-G3′-6190, with very little reaction against the alternative fusion proteins. Fourteen convalescent only sera were available from the second epidemic, eight of which came from patients infected with SHL2 RSV, and the rest from patients in which the genotype of the infecting RSV had not been determined. Twelve of these sera showed strong reactions with GST-G3′-6190, and the remaining two, weak reactions (Table II). In nine of the 12 sera the response was highly restricted to GST-G3′-6190, while in the other three there was a more broad reaction.

In a few instances only, due to the scarcity of the sera, the ELISA titres were determined against the various fusion proteins. For example, baby 69 showed an increase in titre from $<1/200$ in acute serum to $1/3{,}200$ (positive O.D. > 0.2) in the convalescent serum against GST-G3′-6190 (SHL2) while the titres to GST-G3′5857 and GST-G3′-642 remained at $<1/200$.

The specificity of these responses as determined by ELISA tests was confirmed by immunoblotting for nine patients. Figure 4 illustrates four examples, one each for a baby infected with SHL1/3/4 genotype (study no. 86, panel a), SHL2 genotype (study no. 69, panel b), SHL5 genotype (study no. 19, panel c), or SHL6 genotype (study no. 98, panel d) of RSV. In each case, identical quantities of fusion protein were loaded onto each gel, and qualitatively, the relative amounts of the different proteins were approximately the same. The left side of each panel shows the reaction of the acute serum, and the right side shows the paired convalescent serum.

As with the ELISA results, these babies showed a serum antibody response to the fusion proteins which was related to the genotype of the infecting virus. The babies infected with SHL2 or SHL5 genotype RSV showed a highly specific response to the homologous fusion protein but very little response to the other fusion proteins (Fig. 4b,c). In contrast, babies infected with SHL1/3/4 or SHL6 viruses showed a more cross-reactive





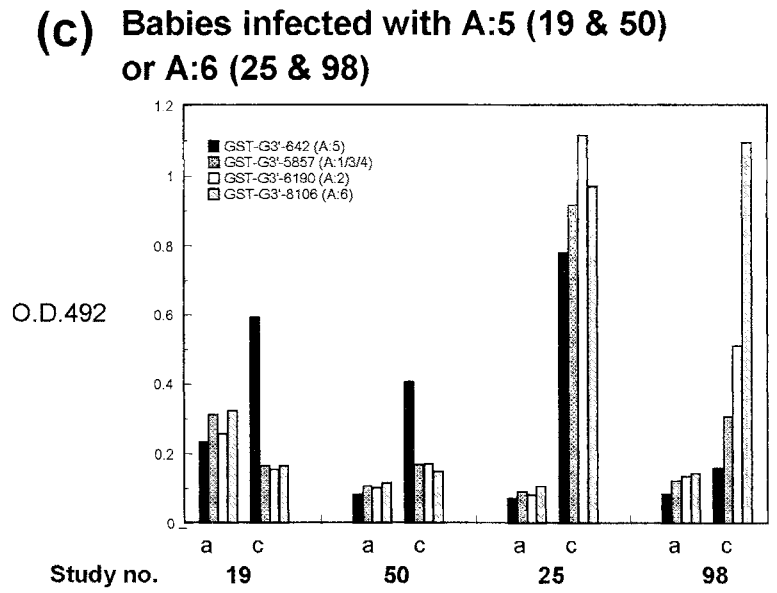
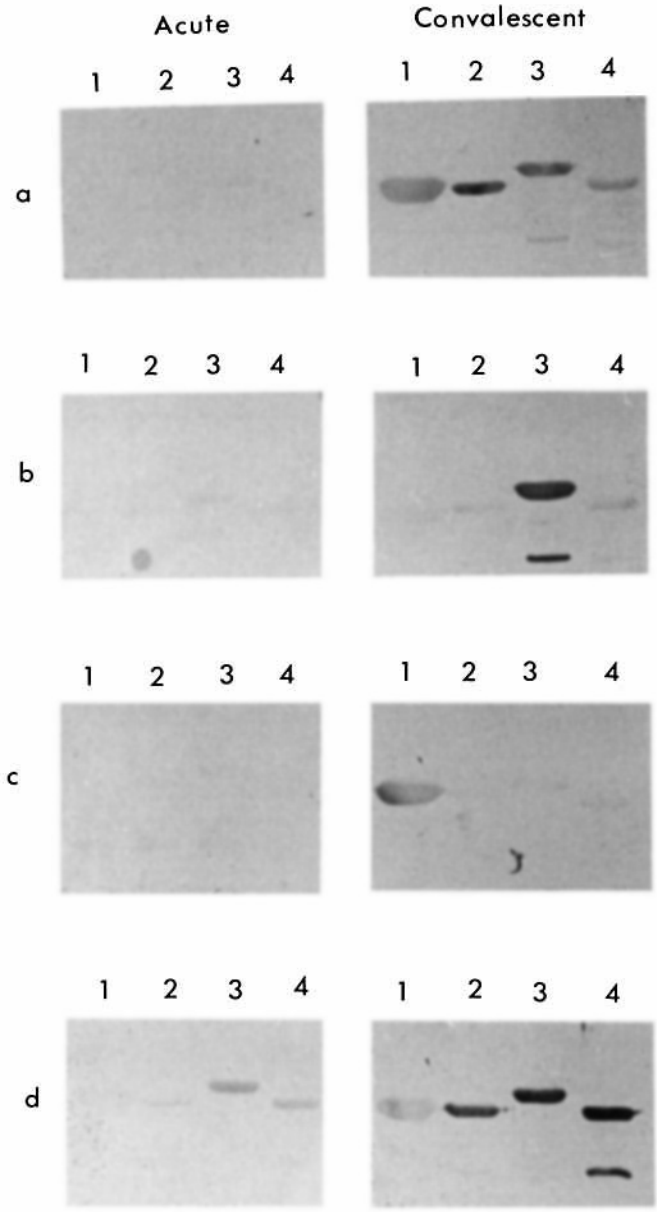


Fig. 3. Comparison by ELISA of reactions of acute and convalescent sera with GST-G fusion proteins. All sera were tested at 1:200 dilution. Results from **(a)** babies infected with SHL1/3/4 genotype RSV, **(b)** babies infected with SHL2 genotype RSV, and **(c)** babies infected with SHL5 (study nos. 19 and 50) or SHL6 (study nos. 25 and 98) genotypes of RSV. a, acute; c, convalescent.



response, particularly to the most closely related fusion proteins (Fig. 4a,d). In the case of baby no. 98, it can be seen that the acute serum also had some reactivity with GST-G3′-6190 and GST-G3′-8106. This could either be residual maternal antibody or the beginnings of the baby's own antibody response.

DISCUSSION

The data presented in this report show that there can be a serological response to the carboxy end of the G protein of RSV during a primary infection in infants older than 7 weeks, and that this can be detected even when the target antigen is expressed as a fusion protein with GST in *E. coli* and lacks glycosylation.

The ability of the babies to respond to the variable part of the G protein appeared to be related to some extent to the age of the baby as has been previously reported by others in relation to the response to affinity purified whole G protein [Murphy et al., 1986a,b]. Since little or no response to the GST fusion proteins was seen in the vast majority of acute sera, this would suggest that there was little or no maternal antibody specific for these GST fusion proteins present in these babies. Thus, the failure of some babies to respond to this variable part of the G protein is unlikely to be due to suppression by pre-existing maternal antibody. An interesting question remaining is whether the lack of maternal antibody directed against this part of the G protein is in any way related to the more severe course of the infection in these hospitalised babies.

Only about 28% (5 of 18) of babies infected with SHL 1/3/4 showed an antibody response to the test antigens whereas 4 of 4 babies infected with SHL2 in the first epidemic showed a response, and the majority of convalescent sera from the second (predominantly SHL2) epidemic showed a strong reaction. The numbers are too small to draw any definite conclusions but these results suggest that certain genotypes may be better at eliciting an antibody response. Complicating factors in such analyses are the ages of the babies and the possibility that maternal antibody is influencing the risk of hospitalisation of the babies.

The specificity of the response to this carboxy-terminal region of the G protein appears to vary with the genotype of the virus, with certain genotypes (SHL1/3/4 and SHL6) inducing a rather broad reaction while SHL2 viruses mostly induce a highly specific reaction. The degree of cross-reaction induced by the SHL1/3/4 and SHL6 genotypes in some babies reflects the relatedness of the G proteins as illustrated in Figure 1. These

Fig. 4. Immunoblot analysis of acute and convalescent sera from four babies. The left-hand panels have been reacted with acute sera and the right-hand panels with convalescent area. All sera were diluted 1:200. **a:** Baby study no. 86, infected with A:SHL1/3/4 genotype. **b:** Baby study no. 69, infected with A:SHL2 genotype. **c:** Baby study no 19, infected with A:SHL5 genotype. **d:** Baby study no. 98, infected with A:SHL6 genotype. **Lanes 1,** GST-G3′-642 (SHL5); **lanes 2,** GST-G3′-5857 (SHL1/3/4); **lanes 3,** GST-G3′-6190 (SHL2); **lanes 4,** GST-G3′-8106 (SHL6).

two genotypes are the most closely related, with SHL2 more distantly related and finally SHL5 being the most distantly related to the other genotypes. Thus, the recognition by the human antibody response mirrors the genetic relatedness of the isolates (see Fig. 4d). Why infections with SHL2 type viruses should produce such a highly specific response is unclear. The G proteins of these viruses share many epitopes as determined by their reactions with murine mabs including some that recognise these fusion proteins (e.g., 021/9G, see above) although they can be distinguished with other mabs [Garcia et al., 1994; Cane and Pringle, 1995]. Likewise, the babies infected with SHL1/3/4 or SHL6 RSV produce antibodies that cross-react with SHL2 derived fusion protein. The specificity of some of these responses could help to explain why it has not been possible previously to find antibodies in human convalescent sera to the variable parts of the G protein.

One aspect of the anti-G response that has not been examined for these babies was their response to whole G protein, which includes conserved regions. This was due to the small amounts of sera available. However, the primary response to whole F and G proteins has been extensively examined by other workers [Murphy et al., 1986a,b], who found that age primarily affected the anti-F response while pre-existing antibody affected the response to the whole G protein, although some of this data may require additional interpretation in view of this demonstration that at least part of the anti-G response may be highly specific to the infecting genotype of RSV. Some non-responding sera in this study were tested against vaccinia expressed G and F proteins, however, and were likewise found to be nonresponsive to these antigens (data not shown).

In conclusion, this report shows that the carboxy-terminal region of the G protein of group A RSV can be recognised by the human antibody response during a primary infection. This may help to explain why this part of the G protein is highly variable and appears to show progressive accumulation of amino acid changes with time [Cane and Pringle, 1995]. It has still to be shown, however, that the antibodies directed against this part of the protein are protective. In addition, it has been demonstrated that the response of the babies is directly related to the genotype of the RSV that infected them. It should be possible, therefore, to determine by this approach the genotype of the infecting virus even in babies for whom no virus isolation is available. This should considerably facilitate the analysis of the molecular epidemiology of the virus in the infants who are not admitted to hospital.

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